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Original Article

Antibacterial Activity of DBD Plasma-Activated Saline Against Clinically Relevant Bacteria: Potential Applications in Family and Reproductive Health

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Abstract

Objective: Effective infection control in family and reproductive healthcare settings is essential for preventing transmission of clinically significant pathogens. Plasma-activated saline (PAS), generated by non-thermal atmospheric-pressure plasma, has emerged as a promising eco-friendly antimicrobial agent due to the generation of reactive oxygen and nitrogen species (RONS). This study evaluated the antibacterial activity of PAS produced by a dielectric barrier discharge (DBD) plasma system and investigated its potential applicability in reproductive and family healthcare environments.

Materials and methods: In this experimental study sterile physiological saline was exposed to a DBD plasma system under atmospheric pressure for different activation durations. Antibacterial activity was evaluated against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella spp*., *Escherichia coli, Staphylococcus aureus*, and *Shigella sonnei*. Bacterial suspensions were treated with PAS at different exposure times, and viable bacterial counts were quantified using the plate count method.

Results: Increasing plasma activation time significantly altered the physicochemical properties of saline, particularly by decreasing pH and increasing reactive nitrogen species concentrations. PAS demonstrated strong time-dependent antibacterial activity against all tested Gram-positive and Gram-negative bacteria. More than 99.9% (>3-log) reduction in viable bacterial counts was achieved after 1 minute of treatment. No detectable bacterial growth was observed after 3 minutes of exposure for Gram-negative bacteria, whereas *Staphylococcus aureus* required 4 minutes for complete inhibition. Statistical analysis demonstrated significant reductions in bacterial survival with increasing treatment duration.

Conclusion: DBD plasma-activated saline exhibited rapid and substantial antibacterial effects against clinically relevant pathogens associated with infection control. Its potent antimicrobial activity, environmental compatibility, and absence of persistent chemical residues highlight its potential as an alternative disinfectant for medical device sterilization, surface decontamination, and hygiene-related applications.

Keywords: Plasma-Activated Saline; Non-Thermal Plasma; Antibacterial Activity; Infection Control; Family Health; Reproductive Health; Reactive Oxygen and Nitrogen Species

Introduction

Healthcare-associated infections (HAIs) remain a major concern in family and reproductive healthcare facilities, particularly among pregnant women, postpartum mothers, neonates, and immunocompromised patients. Infection-related complications such as urinary tract infections,

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puerperal sepsis, neonatal infections, and sexually transmitted bacterial diseases continue to contribute substantially to global morbidity and mortality. Effective and safe disinfection strategies are therefore essential for protecting these vulnerable populations and maintaining hygienic clinical environments. In reproductive healthcare settings, repeated disinfection of gynecological instruments, neonatal care equipment, examination surfaces, and laboratory environments requires antimicrobial approaches that are both effective and environmentally safe. However, conventional chemical disinfectants—including chlorine compounds, aldehydes, alcohol-based agents, and quaternary ammonium salts—present important limitations such as cytotoxicity, mucosal irritation, environmental burden, and the increasing emergence of antimicrobial resistance (AMR) (1-3).

Non-thermal atmospheric pressure plasma (NTAPP) has emerged over the past decade as a transformative antimicrobial technology with expanding biomedical applications, including wound healing, sterilization, oncology, and infection control (4–6). Among plasma-derived technologies, plasma-activated water (PAW) has attracted particular attention due to its ease of application and indirect antimicrobial capability. When plasma interacts with water—especially via dielectric barrier discharge (DBD) systems—it generates a chemically active solution enriched with reactive oxygen and nitrogen species (RONS) (7–9). These species include long-lived components such as hydrogen peroxide (H_2O_2), nitrite (NO_2^-), nitrate (NO_3^-), and ozone (O_3), as well as short-lived radicals such as hydroxyl radicals (OH), superoxide (O_2^-), nitric oxide (NO), and peroxynitrite ($ONOO^-$) (10–12). The synergistic interaction of these reactive species induces oxidative stress, lipid peroxidation, protein oxidation, membrane disruption, and nucleic acid damage, culminating in microbial inactivation.

Importantly, the physicochemical properties of plasma-activated saline—including decreased pH, increased electrical conductivity, total dissolved solids, and accumulation of reactive nitrogen species—are strongly associated with antimicrobial efficacy (13-15). Recent computational modeling studies of dielectric barrier discharge systems have also contributed to understanding plasma temperature distribution, electron density, and reactive species generation mechanisms (16). Such studies provide valuable insight for optimization and reproducibility of plasma-based biomedical applications.

PAW has demonstrated broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria. Gram-negative organisms—including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella* spp., and *Shigella* spp.—possess an outer membrane containing lipopolysaccharides, which can be destabilized by oxidative stress induced by RONS (17-19).

Gram-positive bacteria such as *Staphylococcus aureus*, characterized by a thick peptidoglycan layer, are also susceptible to plasma-derived oxidative mechanisms, although susceptibility patterns may differ due to cell wall structural differences (18, 20). Comparative studies have shown that treatment duration, plasma configuration, activation time, and storage conditions significantly influence bacterial susceptibility.

Moreover, experimental evidence, including our previous investigation, demonstrated substantial reductions in viable cell counts following exposure to DBD-generated PAW (21). These findings align with accumulating literature supporting PAW as a promising antimicrobial platform.

From an environmental and safety perspective, PAW offers significant advantages. It requires only water and electricity for generation, leaves no toxic chemical residues, and decomposes into environmentally benign products, making it suitable for sustainable healthcare systems (22, 23). These characteristics are particularly relevant in family and reproductive health settings, where repeated disinfection of instruments, examination surfaces, delivery environments, and neonatal care equipment is essential.

Despite growing evidence supporting the antimicrobial performance of plasma-activated liquids, their integration into family and reproductive healthcare systems requires further investigation under clinically relevant conditions and standardized plasma parameters. Therefore, the present study aimed to (1) evaluate the antibacterial efficacy of DBD-generated plasma-activated saline against clinically important Gram-positive and Gram-negative bacteria, and (2) characterize the associated physicochemical changes contributing to microbial inactivation. By combining plasma science, microbiology, and infection control research, this study seeks to further explore the translational potential of plasma-activated saline in reproductive healthcare environments.

Materials and methods

1. Plasma System and Experimental Setup

Plasma-activated saline (PAS) was generated using a dielectric barrier discharge (DBD) non-thermal plasma system powered by an alternating high-voltage source operating at 10 kV peak voltage, 20 kHz frequency, and 120 W input power. The discharge configuration consisted of two independent Pyrex glass pipettes immersed in 500 mL of sterile physiological saline contained in a graduated glass cylinder. Each pipette contained a metallic electrode connected to the power supply through insulated cables. Ambient air was separately introduced into each pipette using aquarium air pumps at a flow rate of 4.5 L/min per pipette, generating plasma inside the confined gas phase of the pipettes. Upon plasma generation, filamentary microdischarges formed within the pipettes, producing reactive oxygen and nitrogen species (RONS) that diffused across the gas–liquid interface into the surrounding saline solution.

The lower ends of the pipettes were positioned approximately 1 cm above the bottom of the container to facilitate uniform plasma-liquid interaction. During plasma exposure, the bulk liquid temperature was monitored continuously. The temperature increased gradually from 25 ℃ to a maximum of 34 ℃ after 5 minutes of treatment, confirming the non-thermal nature of the plasma system. The relatively large treatment volume (500 mL) was selected to simulate practical disinfection conditions relevant to healthcare and environmental applications. A schematic illustration of the experimental setup is presented in Figure 1.

2. Physicochemical Characterization of Plasma-Activated Saline

To evaluate the chemical alterations induced by plasma discharge in the aqueous medium, several physicochemical parameters were systematically measured immediately after treatment.

The following parameters were determined for both plasma-treated and untreated control samples:

- **pH:** Monitored using a calibrated NP-345 digital pH meter.
- **Total Dissolved Solids (TDS):** Determined using a TDS-3 SMART handheld hardness meter.
- **Electrical conductivity:** Measured with a NETPIL EZ9908 digital conductivity meter.
- **Nitrate (NO_3^-) and nitrite (NO_2^-):** Determined using specific assay kits supplied by Vaheb Co. (Iran).

3. Microbial Strains and Culture Preparation

The antimicrobial activity of plasma-activated saline (PAS) was assessed using a panel of clinically relevant Gram-positive and Gram-negative bacterial species:

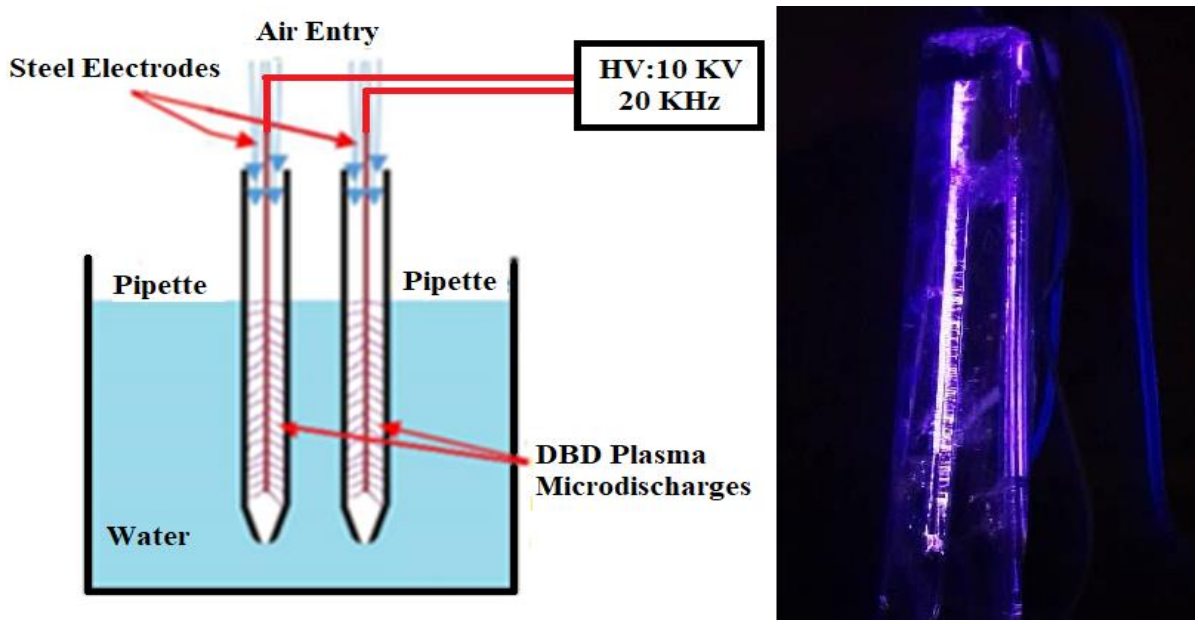

Figure 1: Schematic illustration of the dielectric barrier discharge (DBD) plasma system used for plasma-activated saline generation. The setup consisted of two plasma-generating Pyrex pipettes supplied with atmospheric air and immersed in sterile physiological saline.

- *Staphylococcus aureus* (ATCC 29213)
- *Escherichia coli* (ATCC 25922)
- *Pseudomonas aeruginosa* (ATCC 27853)
- *Klebsiella pneumoniae* (ATCC 13883)
- *Salmonella* spp. (clinical isolate)
- *Shigella sonnei* (clinical isolate)

All bacterial strains were maintained on nutrient agar and sub-cultured prior to each experiment to obtain viable and active cells.

Well-isolated colonies from 24-hour cultures incubated at 37 °C were selected and transferred into 5 mL of sterile physiological saline (0.85% NaCl). The bacterial suspension was adjusted to a turbidity equivalent to the 0.5 McFarland standard. The standardized inocula were prepared immediately prior to plasma treatment and used without delay for antibacterial assays.

4. Determination of Initial Bacterial Load

To quantify baseline bacterial concentration prior to plasma exposure, 100 µL of the standardized bacterial suspension was inoculated into 500 mL of sterile physiological saline contained in a graduated cylinder. Serial ten-fold dilutions were prepared using sterile saline as diluent.

For enumeration, 1 mL from each selected dilution was combined with 9 mL of molten nutrient agar (maintained at approximately 45 °C) and mixed gently before being poured into sterile Petri dishes (pour plate method). Plates were allowed to solidify at room temperature and subsequently incubated at 37 °C for 24 hours. Colony-forming units (CFU/mL) were counted, and plates containing 30–300 colonies were considered for analysis.

5. Plasma Treatment of Bacterial Suspensions

After preparation of the bacterial suspension, the plasma electrodes were directly immersed into the inoculated saline within the graduated cylinder. Plasma treatment was applied for 1, 2, 3, 4 and, 5 minutes under continuous airflow conditions.

At each exposure interval, aliquots were aseptically withdrawn and immediately processed for microbial enumeration using the pour plate technique as described above. Each experimental condition was performed in triplicate to ensure reproducibility.

Untreated bacterial suspensions served as control samples. Reduction in viable bacteria was determined by comparing CFU counts between treated and control groups.

6. Statistical Analysis

All experiments were performed in triplicate, and the results are presented as mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to evaluate differences among plasma treatment times. A p-value < 0.05 was considered statistically significant.

Data processing and statistical analyses were performed using SPSS software. Graphical presentation of the data, including charts and error bars, was prepared using Microsoft Excel.

Results

Physicochemical Characterization of Plasma-Treated Water

The physicochemical properties of the water, including pH, Electrical Conductivity (EC), Total Dissolved Solids (TDS), Nitrate (NO_3^-), and Nitrite (NO_2^-), were analyzed at different time intervals (0, 1, 2, 3, 4, and 5 minutes) following plasma treatment. Statistical analysis, including one-way ANOVA and Tukey's HSD post-hoc test (α=0.05), was performed to determine the significance of observed changes (Table 1).

A statistically significant decrease in pH was observed over time ($p<0.0001$). The pH dropped from an initial mean of 7.78±0.01 at 0 minutes to 5.94±0.06 at 5 minutes.

Tukey's HSD test indicated significant differences between all consecutive time points, with the pH values decreasing progressively (a, b, c, d, e, f, representing distinct statistical groups) (Figure 2).

Table 1: Physicochemical parameters (pH, TDS, EC, nitrate, and nitrite) of plasma-activated water at different treatment times

Parameter	Plasma Treatment Time (min) (Mean ± SD)					
	0	1	2	3	4	5
pH	7.78 ± 0.01	7.27 ± 0.05	6.95 ± 0.02	6.62 ± 0.04	6.27 ± 0.05	5.94 ± 0.06
EC (µS/cm)	655.67 ± 11.52	1015.33 ± 7.92	1243.33 ± 8.50	1420.00 ± 17.61	1631.67 ± 9.46	1818.67 ± 20.18
TDS (ppm)	389.33 ± 4.04	439.33 ± 2.52	481.67 ± 2.52	527.00 ± 5.20	577.00 ± 6.08	651.67 ± 10.12
NO_3^- (mg/L)	0.33 ± 0.58	6.67 ± 1.15	12.00 ± 2.81	16.67 ± 1.47	23.67 ± 2.058	29.33 ± 2.08
NO_2^- (mg/L)	0	0.29 ± 0.08	0.5 ± 0.06	0.79 ± 0.05	0.99 ± 0.07	1.19 ± 0.04

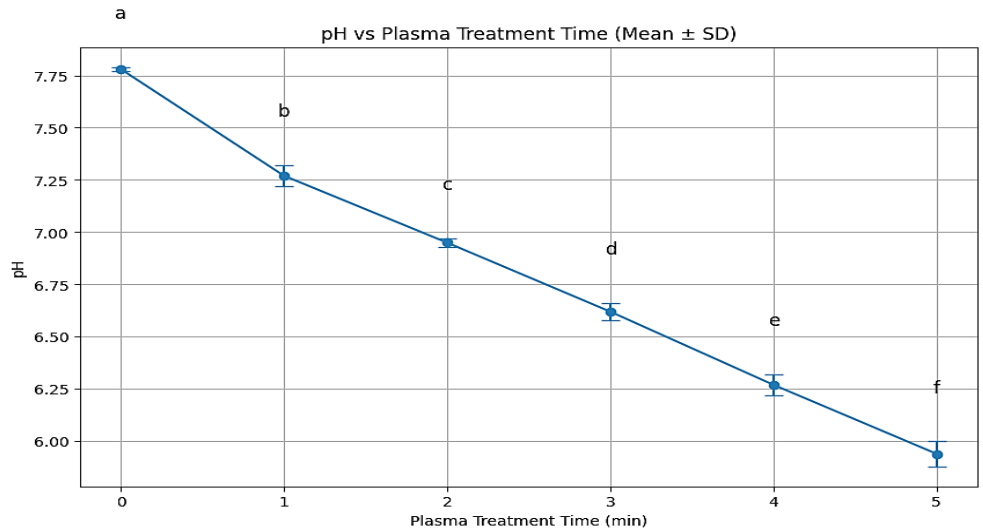

Figure 2: Changes in pH values of plasma-activated saline during different plasma treatment times. Data are presented as mean ± standard deviation (SD) from three independent experiments (n = 3). A significant decrease in pH was observed with increasing plasma exposure time, indicating progressive acidification due to the formation of reactive nitrogen species. Different lowercase letters (a–f) indicate statistically significant differences among treatment groups ($p < 0.05$).

Electrical Conductivity (EC): EC showed a significant increase throughout the treatment duration ($p<0.0001$).

The mean EC rose from 655.67±11.52 µS/cm at 0 minutes to 1818.67±20.18 µS/cm at 5 minutes. All time intervals were statistically distinct from each other, indicating a consistent rise in dissolved ionic content (Figure 3).

Total Dissolved Solids (TDS): TDS levels also demonstrated a significant upward trend with plasma treatment time ($p<0.0001$). The mean TDS increased from 389.33±4.04 ppm at 0 minutes to 651.67±10.12 ppm at 5 minutes, with each time interval showing a statistically significant difference (Figure 4).

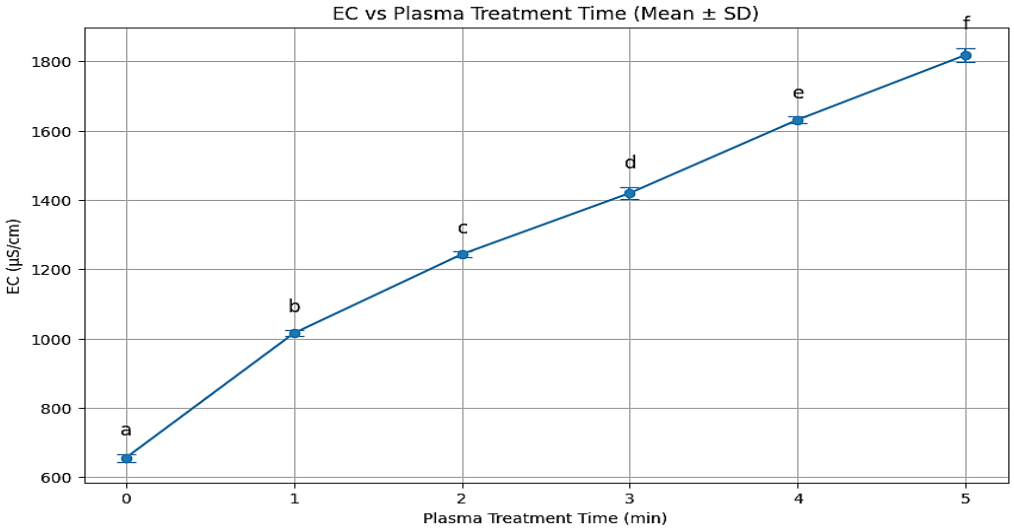

Figure 3: Electrical conductivity (EC) of plasma-activated saline at different plasma treatment durations. Values are expressed as mean ± SD (n = 3). EC increased significantly with increasing plasma exposure time, reflecting the accumulation of plasma-derived ionic species in the saline solution. Different lowercase letters indicate statistically significant differences between treatment groups ($p < 0.05$).

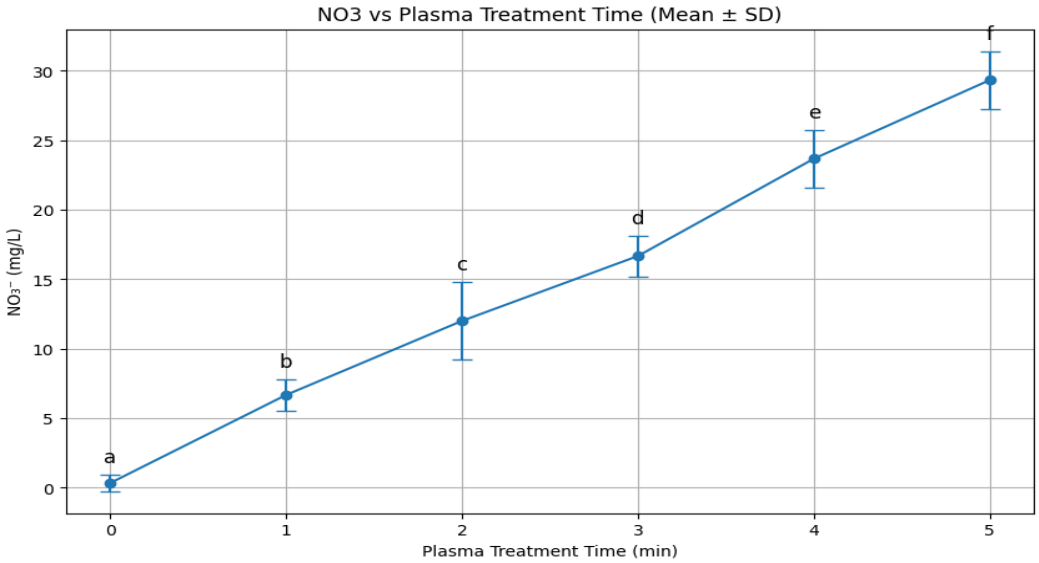

Figure 4: Variations in total dissolved solids (TDS) of plasma-activated saline following plasma treatment. Data are presented as mean ± SD (n = 3). TDS values increased progressively with treatment duration, indicating continuous formation and dissolution of reactive plasma-generated compounds. Different lowercase letters represent statistically significant differences among groups ($p < 0.05$).

Nitrate (NO_3^-): Nitrate concentration exhibited a substantial and statistically significant increase ($p<0.0001$). It rose from a baseline of 0.33±0.58 mg/L to 29.33±2.08 mg/L after 5 minutes of plasma exposure. All sequential time points were found to be significantly different (Figure 5).

Nitrite (NO_2^-): Similarly, nitrite levels increased significantly over the treatment period ($p<0.0001$). Mean nitrite concentration advanced from 0 mg/L at 0 minutes to 1.193±0.04 mg/L at 5 minutes. Significant differences were observed between all consecutive time intervals (Figure 6).

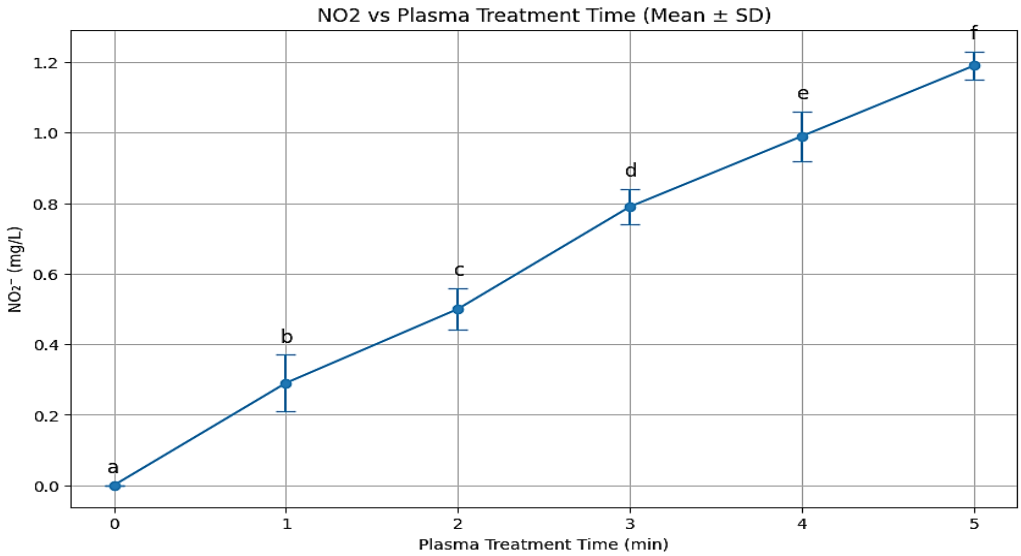

Figure 5: Time-dependent changes in nitrate (NO_3^-) concentration of plasma-activated saline during plasma exposure. Values are shown as mean ± SD (n = 3). Nitrate concentration increased significantly with increasing treatment time due to the generation and dissolution of nitrogen-derived reactive species. Different lowercase letters indicate statistically significant differences among treatment groups ($p < 0.05$).

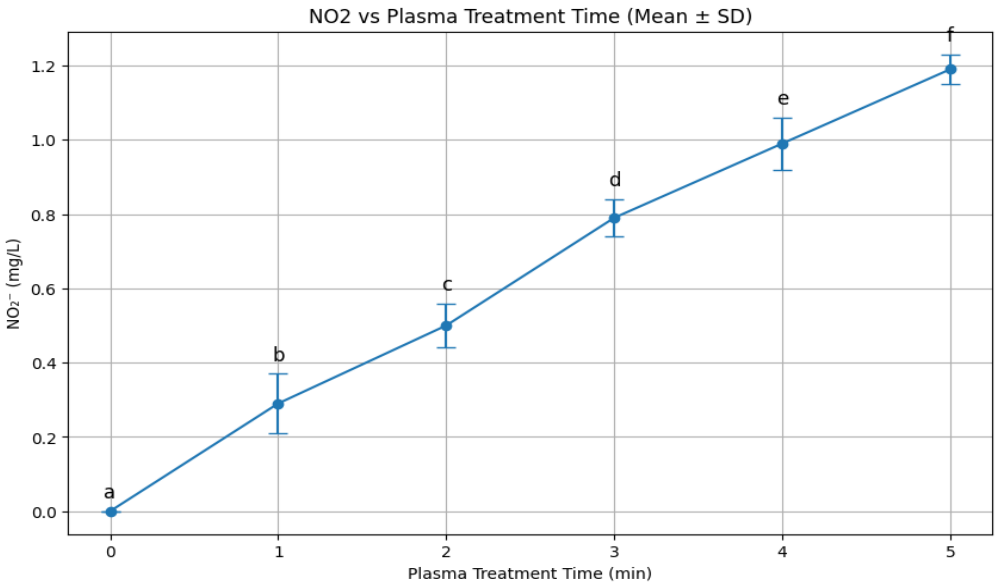

Figure 6: Changes in nitrite (NO_2^-) concentration in plasma-activated saline at different plasma treatment times. Data are expressed as mean ± SD from three independent experiments (n = 3). Nitrite accumulation increased progressively with plasma exposure duration, confirming the formation of reactive nitrogen intermediates associated with antimicrobial activity. Different lowercase letters indicate statistically significant differences among groups ($p < 0.05$).

Bacterial Enumeration: The antibacterial efficacy of plasma-activated saline (PAS) was evaluated against six clinically important bacterial species including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp.*, *Klebsiella pneumoniae*, and *Shigella sonnei*. The initial bacterial concentrations in untreated control samples ranged from approximately 1.17×10^6 to 1.36×10^6 CFU/mL, confirming the high microbial load prior to plasma exposure (Figure 7).

A substantial reduction in viable bacterial counts was observed immediately following PAW treatment. After only 1 minute of exposure, all bacterial species exhibited dramatic decreases in colony-forming units, corresponding to microbial reductions greater than 99.9% (>3-log reduction).

Specifically, *Shigella sonnei* and *Escherichia coli* showed the highest susceptibility to PAW treatment, with bacterial counts decreasing from 1.34×10^6 and 1.27×10^6 CFU/mL to 7.10×10^2 and 7.56×10^2 CFU/mL, respectively.

Similarly, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Salmonella spp.* demonstrated pronounced sensitivity to PAW, reaching reductions of approximately 3 log cycles after 1 minute of treatment. In contrast, *Staphylococcus aureus* exhibited relatively higher resistance compared to Gram-negative species, although a significant decrease from 1.17×10^6 to 1.01×10^3 CFU/mL was still achieved during the first minute of exposure.

Following 2 minutes of treatment, viable bacterial counts approached complete elimination levels in all tested microorganisms. The remaining bacterial populations were extremely low, ranging between 10 and 60 CFU/mL. Complete bacterial inactivation was achieved after 3 minutes of plasma treatment for all tested Gram-negative bacteria, whereas *Staphylococcus aureus* required 4 minutes to reach complete elimination (Figure 8).

Statistical analysis demonstrated highly significant differences between treatment intervals for all bacterial species ($p < 0.0001$). Tukey's post hoc analysis confirmed that each increase in plasma exposure time resulted in a statistically significant reduction in bacterial viability. The obtained results indicate a strong time-dependent antimicrobial activity of PAW against both Gram-positive and Gram-negative bacteria (Table 2).

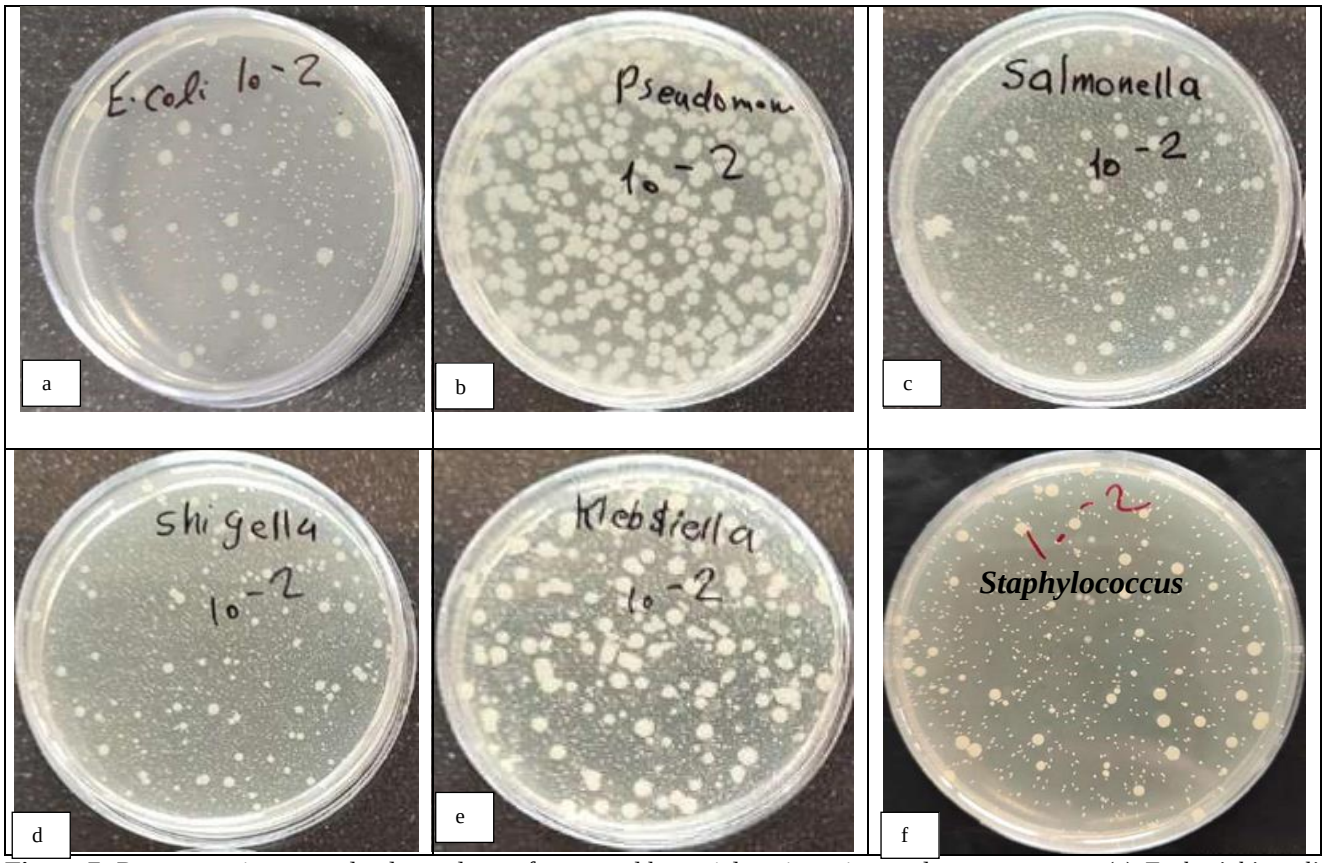

Figure 7: Representative control culture plates of untreated bacterial strains prior to plasma treatment: (a) *Escherichia coli*, (b) *Pseudomonas aeruginosa*, (c) *Salmonella spp*., (d) *Shigella sonnei*, (e) *Klebsiella pneumoniae*, and (f) *Staphylococcus aureus*.

Furthermore, the antimicrobial activity strongly correlated with physicochemical modifications induced in PAS during plasma activation. Increased concentrations of nitrate and nitrite together with progressive acidification of the solution likely contributed synergistically to bacterial inactivation.

Discussion

The present findings demonstrate that plasma-activated saline possesses strong antibacterial activity against both Gram-negative and Gram-positive bacteria. The observed rapid reduction in bacterial viability suggests that the antimicrobial effect is primarily driven by the accumulation of reactive oxygen and nitrogen species generated during plasma activation. These species likely include hydrogen peroxide, nitrite, nitrate, hydroxyl radicals, and other short-lived oxidative intermediates, all of which can disrupt bacterial membranes, proteins, and nucleic acids (24, 25).

The physicochemical changes observed in PAS are consistent with previous reports on plasma–liquid interactions. The decrease in pH together with increased electrical conductivity, total dissolved solids, and elevated concentrations of nitrate and nitrite indicates the formation and accumulation of reactive nitrogen species in the treated solution (26, 27). These conditions create an environment unfavorable for bacterial survival and proliferation.

In this study, Gram-negative bacteria appeared to be generally more susceptible than Gram-positive bacteria, although all tested organisms were significantly affected by PAW. This difference may be explained by structural variations in the bacterial cell envelope. Gram-negative bacteria possess a thinner peptidoglycan layer and an outer membrane that may be more vulnerable to oxidative disruption once reactive species penetrate the cell surface. In contrast, the thicker peptidoglycan layer of *Staphylococcus aureus* may provide somewhat greater resistance, which is consistent with the slightly longer exposure time required for complete inactivation.

The rapid antibacterial action observed here is particularly important from the perspective of family and reproductive health.

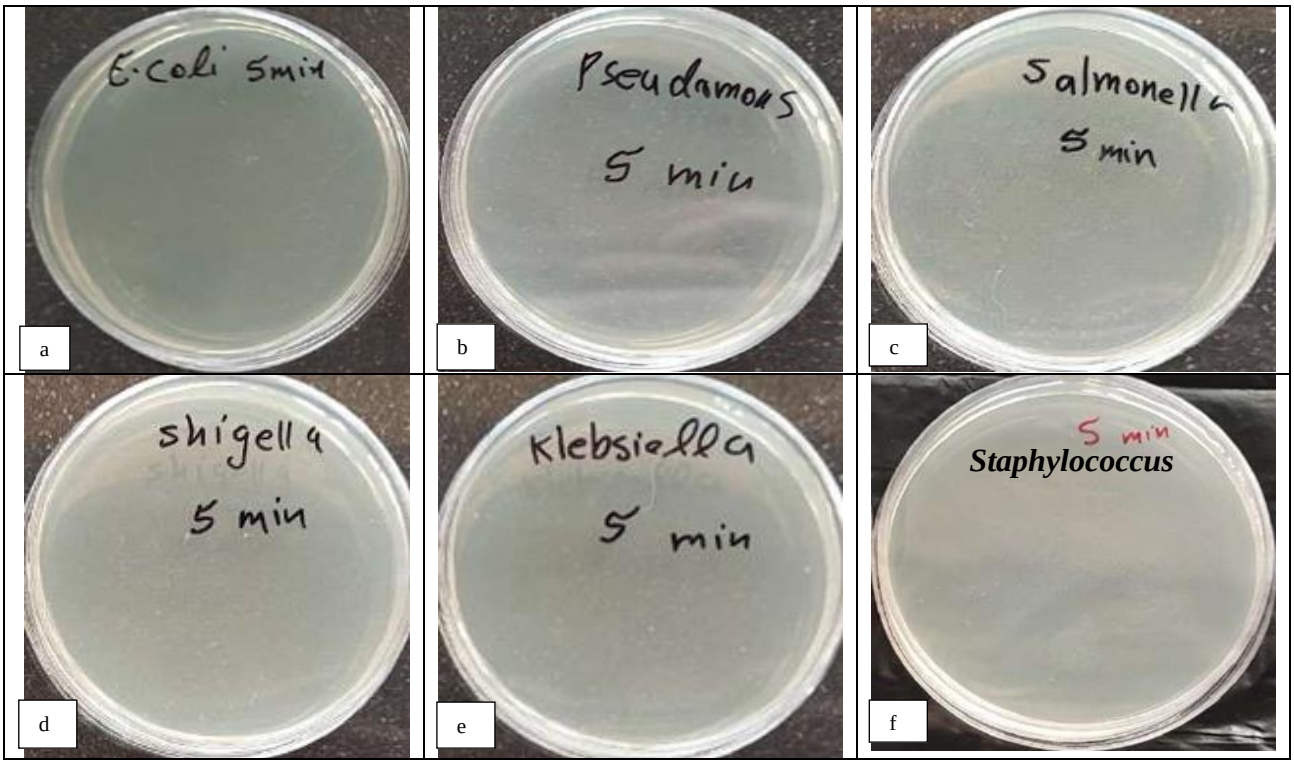

Figure 8: Representative bacterial culture plates after plasma-activated saline treatment: (a) *Escherichia coli*, (b) *Pseudomonas aeruginosa*, (c) *Salmonella spp*., (d) *Shigella sonnei*, (e) *Klebsiella pneumoniae*, and (f) *Staphylococcus aureus*. No detectable bacterial growth was observed after 5 minutes plasma treatment.

Effective and non-toxic disinfection strategies are essential in healthcare environments where chemical residues, antimicrobial resistance, and environmental safety are major concerns. PAS may offer a promising alternative to conventional disinfectants because it can be generated on demand, leaves no harmful chemical residue, and exerts potent antimicrobial effects within a short time. These characteristics make it attractive for potential applications in surface disinfection, instrument decontamination, and hygiene-related interventions in family and reproductive health settings (20, 21, 24, 25).

Another important finding is that complete or near-complete bacterial inactivation was achieved within a few minutes of treatment. This suggests that PAS may be suitable for rapid disinfection protocols where time efficiency is critical. However, the exact antimicrobial performance of PAS may depend on plasma source, activation time, water composition, storage conditions, and the target microorganism. Therefore, optimization of plasma parameters will be necessary before clinical or practical implementation.

Overall, the results support the hypothesis that PAS is an effective antibacterial agent with potential utility in infection control. The combination of physicochemical activation and broad-spectrum microbial inactivation highlights its promise as a safe, eco-friendly, and efficient disinfectant. Further studies should evaluate its stability over time, mechanism of action at the molecular level, and efficacy under real-world conditions relevant to family and reproductive health care.

Table 2: Reduction in viable bacterial counts following plasma-activated saline treatment at different exposure times

Bacterial Species	Initial count (CFU/mL) Mean ± SD	Count after 1 min treatment (CFU/mL) Mean ± SD	Log reduction after 1 min	Time required for no detectable growth
Pseudomonas aeruginosa	$(1.36\pm0.06)\times10^{6}$	$(1.04\pm0.07)\times10^{3}$	3.11	3 min
Staphylococcus aureus	$(1.17\pm0.04)\times10^{6}$	$(1.01\pm0.04)\times10^{3}$	3.06	4 min
Escherichia coli	$(1.27\pm0.11)\times10^{6}$	$(7.56\pm0.05)\times10^{2}$	3.22	3 min
Salmonella spp.	$(1.36\pm0.09)\times10^{6}$	$(1.26\pm0.08)\times10^{3}$	3.03	3 min
Klebsiella pneumoniae	$(1.22\pm0.12)\times10^{6}$	$(9.86\pm0.32)\times10^{2}$	3.09	3 min
Shigella sonnei	$(1.34\pm0.08)\times10^{6}$	$(7.10\pm0.24)\times10^{2}$	3.27	3 min

Limitations: This study has several limitations. First, only planktonic bacterial cultures were evaluated, while biofilm-associated microorganisms were not investigated. Second, the cytotoxicity and biocompatibility of plasma-activated saline were not assessed in mammalian cells or clinical tissues. In addition, the long-term stability of reactive species in plasma-activated saline during storage was not evaluated. Future studies should investigate these aspects to further support the clinical translation and practical application of plasma-activated saline in healthcare environments.

Conclusion

The present study demonstrated that plasma-activated saline generated by a dielectric barrier discharge plasma system possesses strong antibacterial activity against clinically relevant Gram-positive and Gram-negative bacteria. Plasma treatment induced significant physicochemical modifications in saline, including decreased pH and increased electrical conductivity, total dissolved solids, nitrate, and nitrite concentrations, which were associated with marked reductions in bacterial viability.

The antimicrobial activity of plasma-activated saline was rapid, broad-spectrum, and strongly dependent on treatment duration. More than 99.9% bacterial reduction was achieved after only 1 minute of treatment, while no detectable bacterial growth was observed after longer exposure times.

These findings suggest that plasma-activated saline may serve as a promising eco-friendly disinfectant for infection control applications, particularly in family and reproductive healthcare settings where safe and effective antimicrobial strategies are critically needed. Its ability to rapidly inactivate bacteria without leaving persistent chemical residues highlights its potential as an alternative to conventional disinfectants. Nevertheless, additional investigations are required to evaluate cytotoxicity, long-term stability, biofilm inactivation, and practical clinical applicability under real-world conditions.

Conflict of Interests

Authors declare no conflict of interests.

Acknowledgments

None.

References

1. World Health Organization. Future surveillance for epidemic and pandemic diseases: a 2023 perspective. *World Health Organization*; 2023 Dec 11. Available at: https://www.who.int/publications/i/item/9789240080959 .
2. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629-655. Epub 2022 Jan 19. Erratum in: Lancet. 2022;400(10358):1102.
3. Yemiş F, Harmancı NY. Classification, uses and environmental implications of disinfectants. *Pakistan Journal of Analytical & Environmental Chemistry*. 2020;21(2):179-92.
4. Laroussi M. Cold plasma in medicine and healthcare: *The new frontier in low temperature plasma applications. Frontiers in Physics*. 2020;8:74.
5. Privat-Maldonado A, Deben C. Model Systems in Plasma Biomedicine. *In Redox Biology in Plasma Medicine 2024 Jul 12* (pp. 188-201). CRC Press.
6. Khanikar RR, Bailung H. Cold atmospheric pressure plasma technology for biomedical application. *In Plasma science and technology 2021 Oct 29*. IntechOpen.
7. Wong KS, Chew NS, Low M, Tan MK. Plasma-activated water: Physicochemical properties, generation techniques, and applications. *Processes*. 2023;11(7):2213.
8. Mazandarani A, Goudarzi S, Jafarabadi M, Azimi Nekoo E. Effects of Cold Plasma on Staphylococcus Aureus. J Family Reprod Health. 2022;16(3):212-216.
9. Zhang X, Li H, Zeng Y, Hu S, Zhong J, Liu T, et al. Atmospheric Pressure Array Air Plasma Jet for Plasma Activated Water Generation and Its Application for E. *Coli Inactivation. In Chinese Conference on High Voltage and Discharge Plasmas 2024 Nov* 29 (pp. 459-473). Singapore: Springer Nature Singapore.
10. Zhou R, Zhou R, Wang P, Xian Y, Mai-Prochnow A, Lu X, et al. Plasma-activated water: generation, origin of reactive species and biological applications. *Journal of Physics D: Applied Physics*. 2020 Jul 22;53(30):303001.
11. Cross CE, Motchnik PA, Bruener BA, Jones DA, Kaur H, Ames BN, et al. Oxidative damage to plasma constituents by ozone. *FEBS letters*. 1992;298(2-3): 269-72.
12. Kaushik NK, Ghimire B, Li Y, Adhikari M, Veerana M, Kaushik Net al. Biological and medical applications of plasma-activated media, water and solutions. Biol Chem. 2018;400(1):39-62.
13. Thirumdas R, Kothakota A, Annapure U, Siliveru K, Blundell R, Gatt R, et al. Plasma activated water (PAW): Chemistry, physico-chemical properties, applications in food and agriculture. *Trends in food*

science & technology. 2018;77:21-31.
14. Gongora K, Vankerschaver J, Sampers I, Van Haute S. The influence of pH on the efficacy of oxidation-reduction potential (ORP) to predict chlorine disinfection of surrogate bacteria, *Escherichia coli* O157: H7 and Listeria monocytogenes in oxidant demand free conditions and fresh produce wash water. *Food Microbiology*. 2024;121:104516.
15. Vlad IE, Anghel SD. Time stability of water activated by different on-liquid atmospheric pressure plasmas. *Journal of electrostatics*. 2017;87:284-92.
16. Mazandarani A, Goudarzi S, Ghafoorifard H, Eskandari A, Shahshenas S. Calculation of temperature and density for dielectric-barrier discharge (DBD) plasma using COMSOL. *Journal of Nuclear Science, Engineering and Technology*. 2020;40(4):99-108.
17. Mai-Prochnow A, Clauson M, Hong J, Murphy AB. Gram positive and Gram negative bacteria differ in their sensitivity to cold plasma. *Scientific reports*. 2016;6(1):38610.
18. Vega S, Dowzicky MJ. Antimicrobial susceptibility among Gram-positive and Gram-negative organisms collected from the Latin American region between 2004 and 2015 as part of the Tigecycline Evaluation and Surveillance Trial. Ann Clin Microbiol Antimicrob. 2017;16(1):50.
19. Ma R, Wang G, Tian Y, Wang K, Zhang J, Fang J. Non-thermal plasma-activated water inactivation of food-borne pathogen on fresh produce. *Journal of hazardous materials*. 2015;300:643-51.
20. Dolezalova E, Lukes P. Membrane damage and active but nonculturable state in liquid cultures of *Escherichia coli* treated with an atmospheric pressure plasma jet. *Bioelectrochemistry*. 2015;103:7-14.
21. Mazandarani A, Jabbari M, Goudarzi S, Javad Khodashenas M. Evaluating the Efficacy of Plasma-Activated Water in the Elimination of Pathogenic Bacteria: An Experimental Study on Pseudomonas, Salmonella, Escherichia Coli, and Staphylococcus Aureus. J Family Reprod Health. 2025;19(3):167-173.
22. Bruggeman PJ, Kushner MJ, Locke BR, Gardeniers JG, Graham WG, Graves DB, Hofman-Caris RC, Maric D, Reid JP, Ceriani E, Fernandez Rivas D. Plasma–liquid interactions: a review and roadmap. *Plasma sources science and technology*. 2016;25(5):053002.
23. Fernandes FA, Rodrigues S. Cold plasma technology for sustainable food production: meeting the United Nations sustainable development goals. *Sustainable Food Technology*. 2025;3(1):32-53.
24. Rathore V, Patel D, Butani S, Nema SK. Investigation of physicochemical properties of plasma activated water and its bactericidal efficacy. *Plasma Chemistry and Plasma Processing*. 2021;41(3):871-902.
25. Madkour LH. Function of reactive oxygen species (ROS) inside the living organisms and sources of oxidants. *Pharm. Sci. Anal. Res*. J. 2019;2:180023.
26. Pal P, Pal UN, Singh V, Sharma NK, Singh M, Mishra A, Maurya S, Lamba RP. Experimental investigation for the generation and characterization of plasma-activated water. *IEEE Transactions on Plasma Science*. 2024;52(6):2137-43.
27. Pandey S, Jangra R, Ahlawat K, Mishra R, Mishra A, Jangra S, Prakash R. Selective generation of nitrate and nitrite in plasma activated water and its physicochemical parameters analysis. *Physics Letters A*. 2023;474:128832.

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